produce large amounts of interferon-gamma (IFN-g) under inflammatory conditions. Here we address the role of IFN-g production by Tregs in the course of experimental GvHD.

Methods: In a C57BL/6 into BALB/c mouse model of acute GvHD we monitored the intracellular cytokine expression of Tregs using FoxP3-reporter mice (C56BL/6) and congenic markers. We addressed the role of IFN-g in experimental GvHD i) by employing the IFN-g blocking mAb XMG1.2 and ii) by adoptive transfer of Tregs and/ or effector T cells purified from ifng–/– mice. GvHD severity was monitored by survival, clinical score and histological analysis.

Results: Co-transferred Tregs in a C57BL/6 into BALB/c model readily secreted IFN-g but stably remained FoxP3+ and prevented lethal GvHD. Intracellular staining revealed that at day 4 after transplantation approximately 35% of these allogeneic Tregs produced IFN-g. In this experimental setting blocking of IFN-g with mAb completely abolished the protective effect of Tregs and led to early death from exacerbated GvHD. Of note, we also observed a similar fatal outcome of experimental GvHD when we co-transferred ifng–/– Tregs and wild type effector T cells.

Conclusions: Our data suggest that IFN-g should not be regarded as an adverse pro-inflammatory cytokine under the highly inflammatory environment of acute GvHD since it is required for the protective action of Tregs. We hypothesize that this may be due to a deviation of effector T cell profiles towards Th17. In ongoing experiments we therefore address this issue by employing effector T cells from il17a–/– / il17f–/– double knock-out mice.

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DONOR-LYMPHOCYTE INFUSION IN PATIENTS WITH PERSISTENT OR RECURRENT MULTIPLE MYELOMA AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Objective: The graft-versus-leukemia (GVL) effect of donor-lymphocyte infusions (DLI) is well documented in hematologic malignancies, including multiple myeloma (MM). We evaluated the role of DLI in persistent or recurrent MM after allogeneic hematopoietic stem cell transplantation (allo HCT).

Materials and Methods: We identified 23 patients with MM, who received DLI at UT-MD Anderson Cancer Center between 7/1996 -6/2008. Patients had persistent or recurrent disease after allo HCT, and were treated with DLI collected from their original allo HCT donors.

Results: Nineteen patients received DLI from matched related donors (MRD) and 4 from matched unrelated donors (MUD). Median age at DLI was 51 years (range: 38-62). A total of 33 DLI doses were administered. Seventeen patients received 1 infusion, 4 patients received 2, and 2 patient received 4 infusions. Median interval between allo HCT and the first DLI was 8.2 months (range: 2.9 to 119.5). Median follow up from the first DLI was 18 months (range 1-126). Twenty-six DLI (78%) were given without preceding cytoreductive chemotherapy. The median DLI dose was 3.5 x 10^7 CD3+ T cells (range 0.5 to 14.8 x 10^7). Overall response rate was 30%, with 10 of 33 DLI doses associated with objective clinical responses (3 CR, 5 VGPR, 2 PR). Thirteen (39%) additional DLI were followed by stable disease. Median response duration was 5 months (range 2-10). ORR to DLI in relapsed disease was 10% (2/20), compared to 61% (8/13) for residual disease ($p = 0.0048$). Grade II-IV acute graft-versus-host disease (GVHD) was seen in 6 (26%) patients, with median onset of 5 weeks from DLI. None of the patients developed chronic GVHD after DLI. Median overall survival from the first DLI was 18.6 months.

Conclusions: DLI is associated with objective clinical responses patients with relapsed or persistent MM, with a significantly higher response rate in patients with persistent vs. relapsed disease. The risk of acute GVHD was low. The role of DLI needs to be further explored in prospective clinical trials for patients with relapsed or persistent MM.

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KINETICS OF MURINE GVHD INDUCTION ACROSS MINOR AND MAJOR HISTOCOMPATIBILITY BARRIERS

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It is known that Graft-vs-Host Disease (GVHD) occurs in response to minor histocompatibility antigens, but little is known about the kinetics of donor T cell proliferation and homing in minor mismatch models of allogeneic hematopoietic cell transplantation (HCT). This is in contrast to models across major histocompatibility barriers, where early development of GVHD has been better characterized. Yet, because minor mismatch models are more similar to clinical HCT, it is critical to understand how GVHD develops across minor barriers. To investigate temporal and spatial events of donor T cell activation and homing, side-by-side transplants were conducted using bone marrow and enriched CD4 and CD8 T cells (Tcon) from donor C56BL/6 mice ($H2^b$) into either major mismatched Balb/c ($H2^d$), or minor mismatched Balb.b ($H2^b$), irradiated recipients. Balb/c mice received $1x10^6$ Tcon while Balb.b mice received $15x10^6$ Tcon, based on titration experiments. Proliferation and migration of donor Tcon was monitored using *in vivo* and *ex vivo* bioluminescent imaging, and CFSE labeling. Expression of T cell activation and homing markers was examined using flow cytometry analysis of donor CD4 and CD8 cells re-isolated from transplanted mice. Donor Tcon from Balb.b mice exhibited significantly reduced proliferation at both 3 and 6 days post transplant ($p < 0.01$, $n = 43$). But, mirroring our earlier findings in major mismatch models, donor Tcon in the minor model homed to nodal sites by day 3, followed by an exit to tissues by day 6, albeit reduced. No significant differences in the expression of the activation and homing markers examined were noted by day 3, although there was variation across tissues and between CD4 and CD8 cells. By day 6, donor T cells re-isolated from Balb.b recipients had reduced levels of $\alpha_4\beta_7$ and P-selectin, and increased retention of CD62L. These data support the idea that early events of donor T cell activation, particularly spatially, are similar across minor and major histocompatiblity barriers, reinforcing the usefulness of both models as translational research tools. More importantly, these data suggest that delays in visible GVHD onset in minor mismatch tranpslants arise from temporal differences in the effector phase of T cell action, rather than delays in the initiation phase. These findings support targeting very early events in T cell activation as the most effective method of reducing GVHD in clinical settings.

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ROLE OF CLINICAL LABORATORY MARKERS OF INFLAMMATION IN ASSESSING CHRONIC GRAFT VERSUS HOST DISEASE (CGVHD) ACTIVITY AND SEVERITY

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Background: cGVHD is a major cause of health problems and mortality after allogeneic HCT. Routine clinical laboratory parameters are established in outcomes monitoring of systemic inflammatory or autoimmune disease, but their value in cGVHD is unknown. To determine the relationship between laboratory markers of